BREATH BIOMARKERS OF RADIATION EXPOSURE

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Objectives

Short-term

- *Identify biomarkers of radiation in breath*
- *Determine dose-effect response*

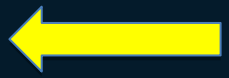
Long-term

- *Migrate findings to a point-of-care platform*

→ *a radiation biodosimeter for first responders*

Background

- *Normal human breath - many volatile organic compounds (VOCs)*
- *Concentrations - low! – picomolar (parts per trillion)*
- *Specialized instruments → detect ~2,000 different VOCs in a sample*
- *Breath VOCs contain biomarkers*

  oxidative stress 

  lung cancer

  breast cancer

  pulmonary TB

  transplant rejection

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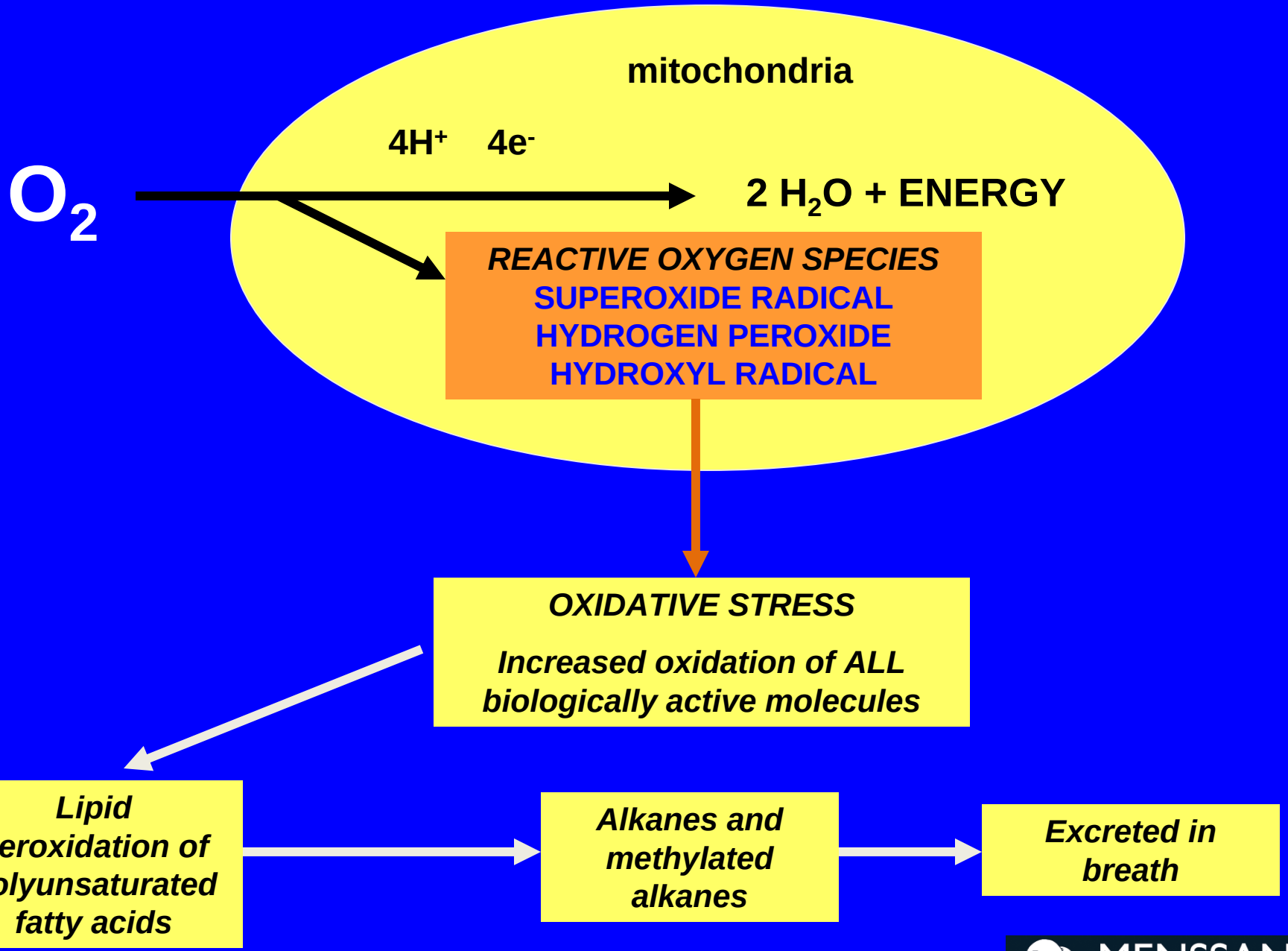

Hypothesis

RADIATION

- → *free radicals in tissues*
- → *oxidative stress*
- → *volatile biomarkers in breath*
  - *alkanes*
  - *alkane derivatives*
  - *other metabolites?*

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Study design

- *Human subjects (n=32)*
  - *Age 18-75*
  - *Treated for lung, prostate or breast cancer*
  - *Daily fractionated regimens for 4 or 5 days*
- *Low-dose group (n=27)*

  Standard radiotherapy

  Daily dose = 180-400 cGy
- *High-dose group (n=5)*

  Stereotactic body radiotherapy (SBRT)

  Daily dose = 700-1200 cGy

Breath sample collections – on days 1, 2, 3, 4, and 5

Before treatment

<30 min after treatment

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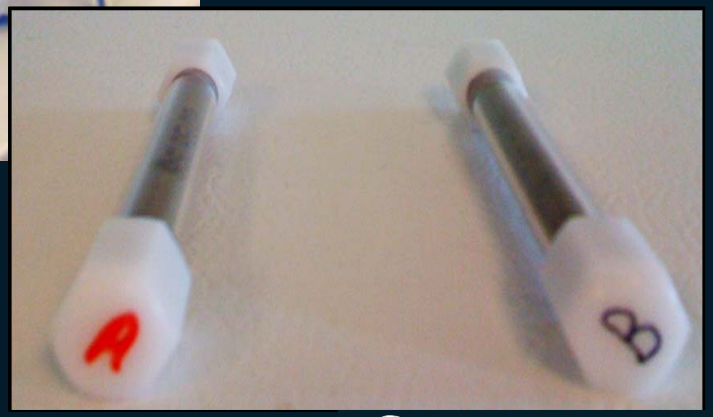
Sample collection

Breath collection apparatus (BCA)



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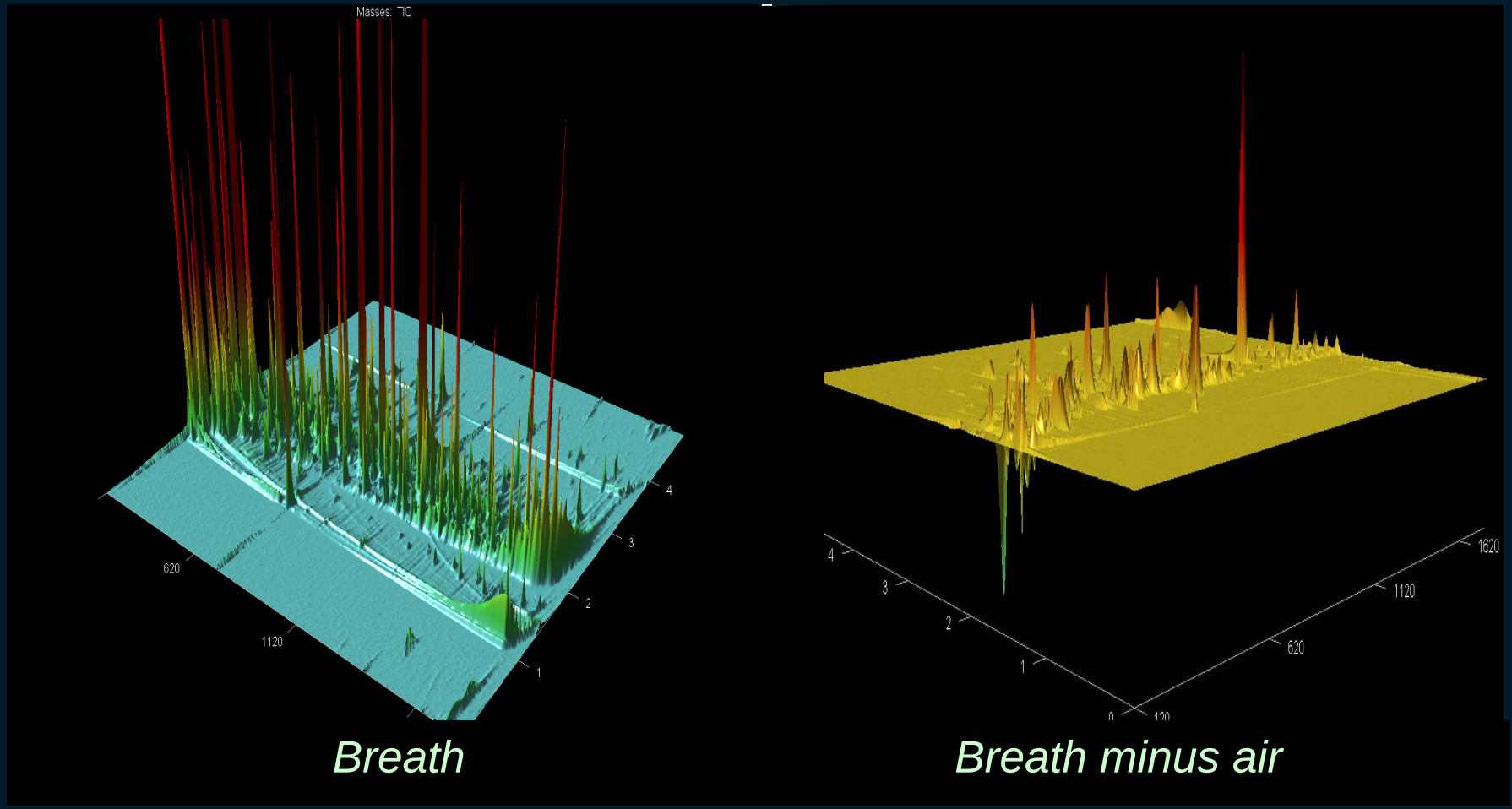
Sample analysis

Two-dimensional gas chromatography and time-of-flight mass spectrometry



Breath

Breath minus air

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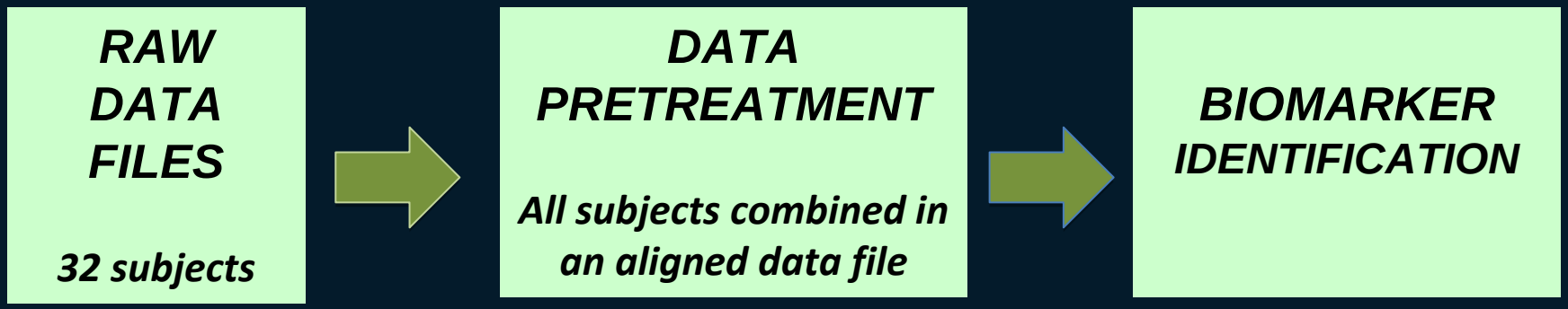
Data analysis



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Data pre-treatment

RAW DATA FILES
GCxGC TOF MS
~ 1 GB each

→

DECONVOLUTE AND IDENTIFY PEAKS
Separate overlapping peaks and match mass spectra with NIST library

→

QUALITY CONTROL
Remove poor matches, contaminants (column bleed)

→

PRIMARY ALIGNMENT
Match peaks by Kovats Index (n-alkane retention times)

→

SECONDARY ALIGNMENT
Match peaks with similar mass spectra

→

NORMALIZE ABUNDANCES
to internal standard

→

DETERMINE ALVEOLAR GRADIENTS
Breath - air VOCS

→

ALIGNED DATA SET
– all subjects

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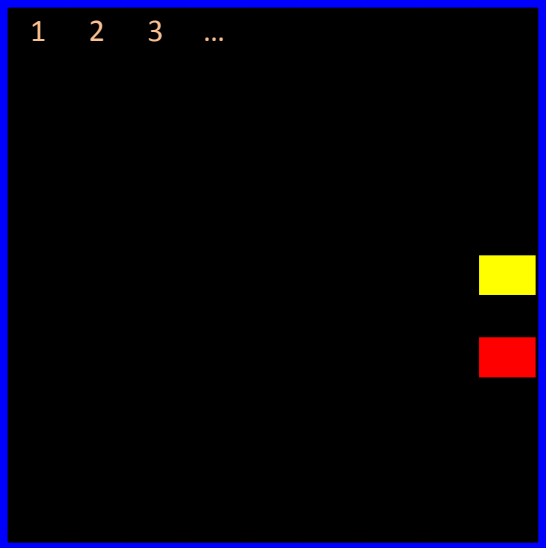
Display aligned data

"Breath chip" heat map

At each time point:

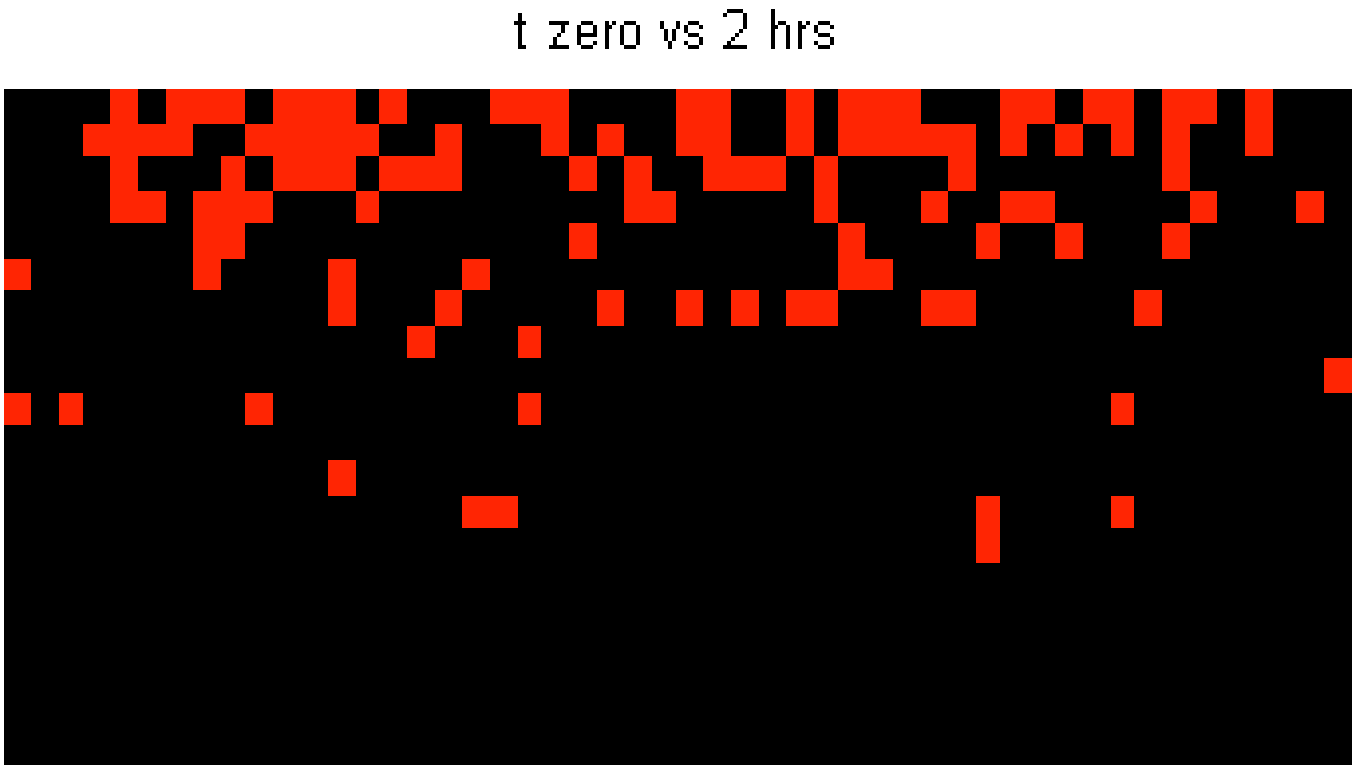
Mean of all subjects
compared to
mean baseline pre-radiation level



Increased

Decreased

Day 1 post-radiation



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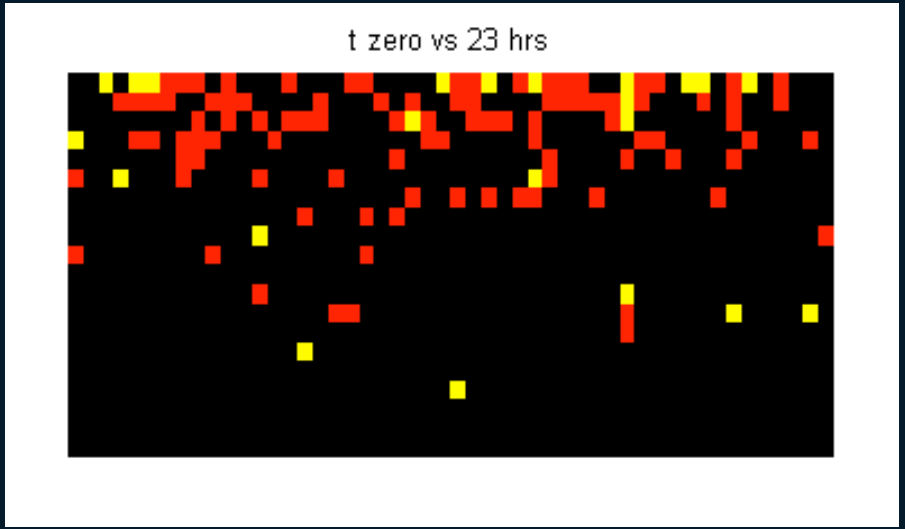
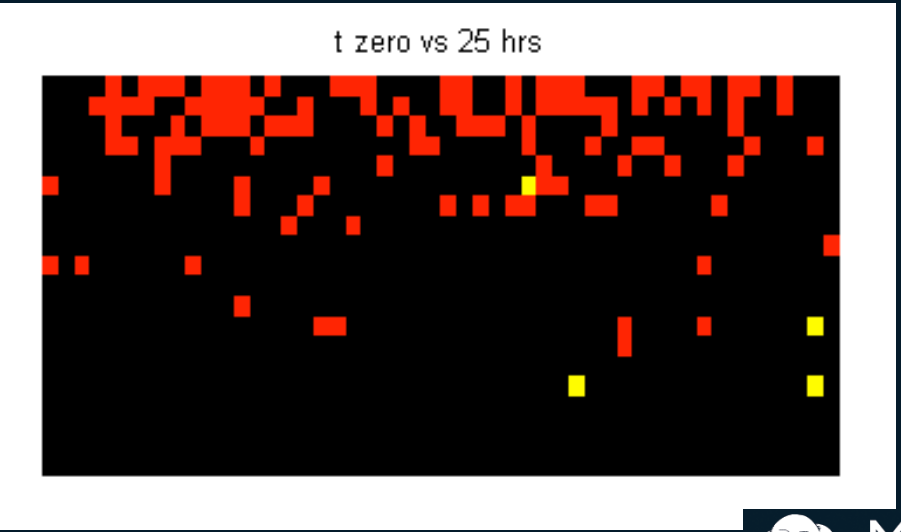
Day 2

Pre-radiation



Post-radiation



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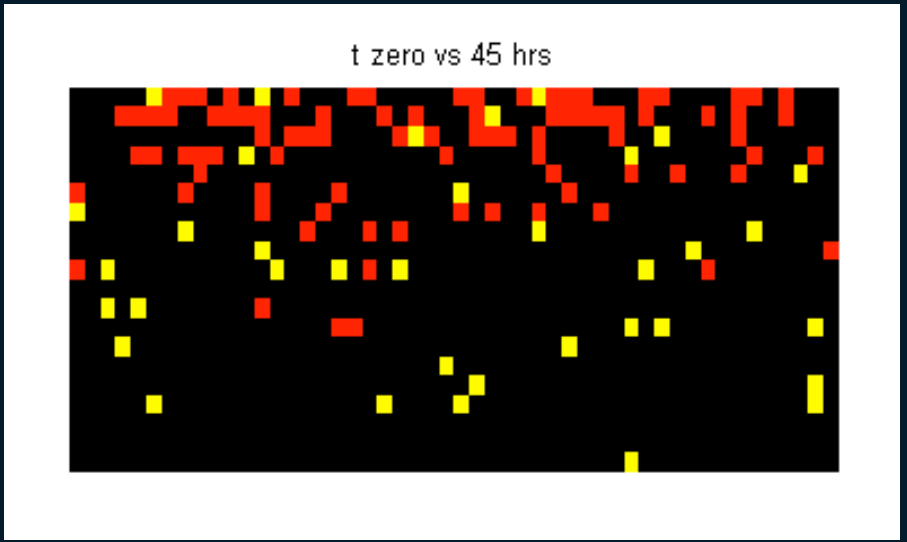
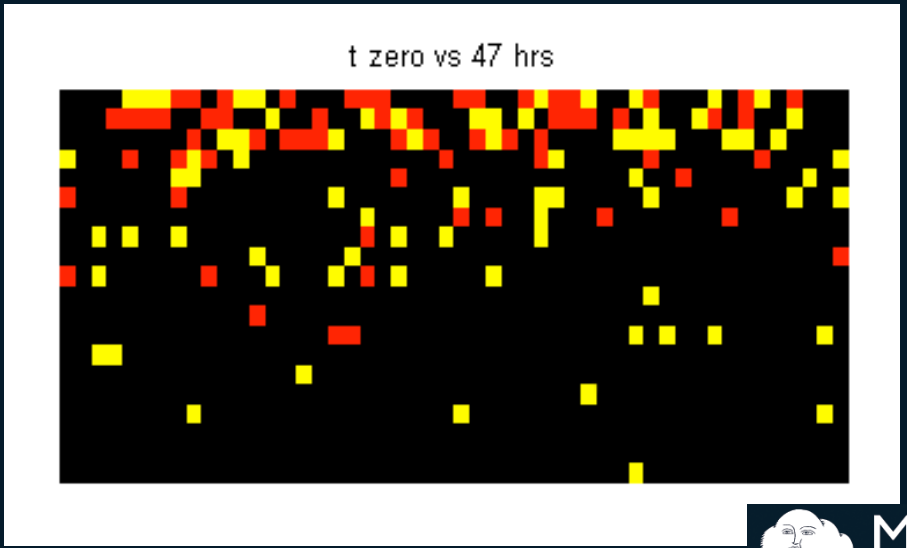
Day 3

Pre-radiation



Post-radiation



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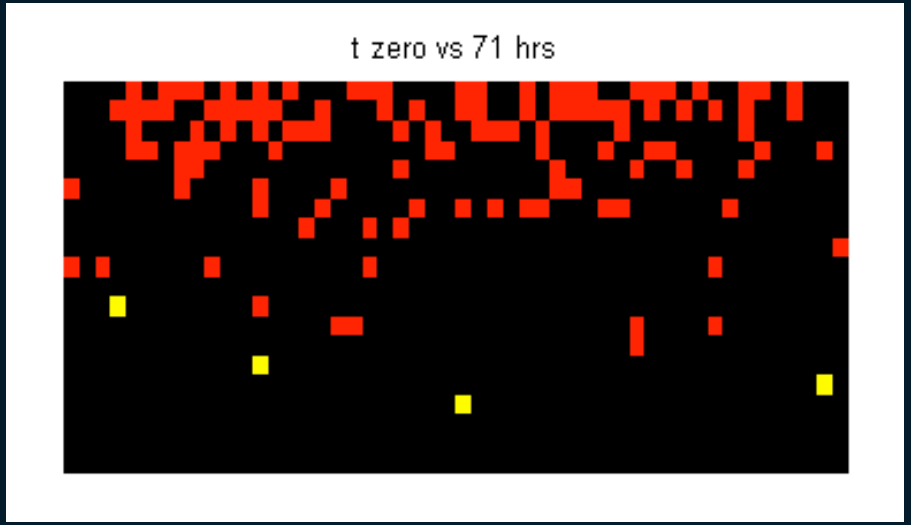
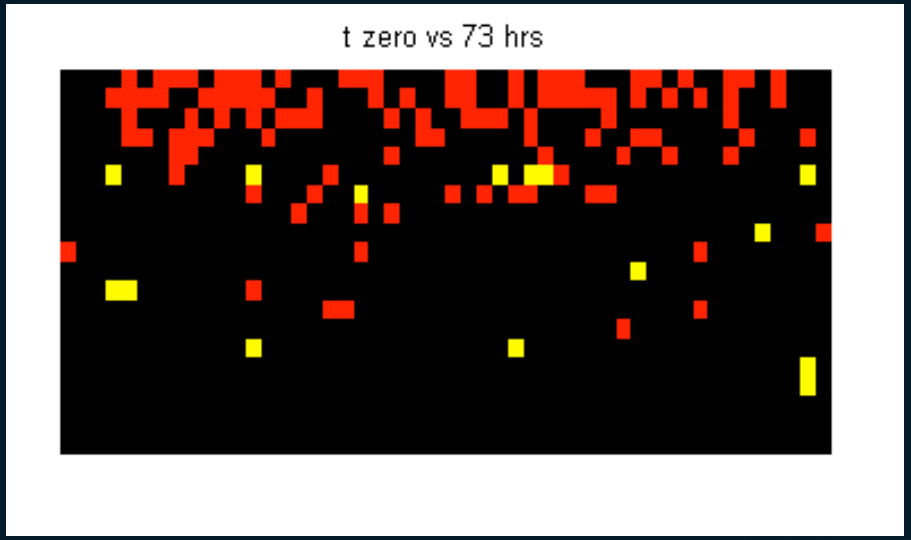
Day 4

Pre-radiation



Post-radiation



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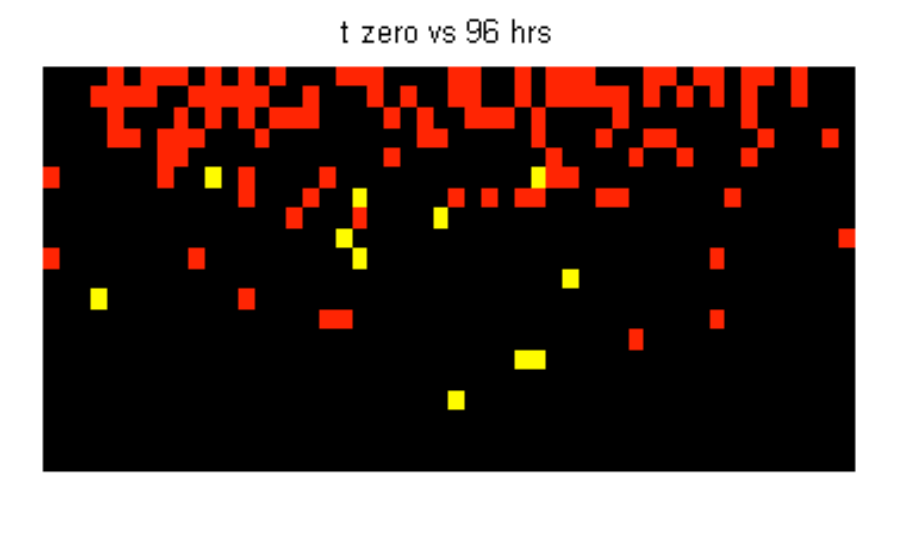
Day 5

Pre-radiation



Post-radiation: only 2 high dose samples – did not align

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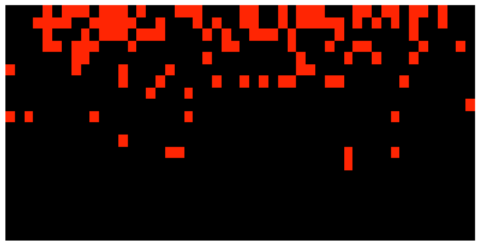
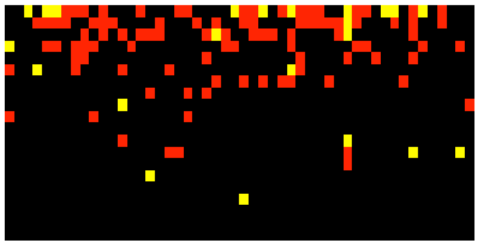
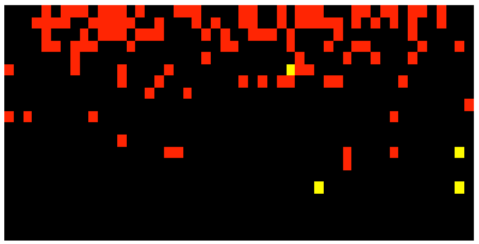
t zero vs 2 hrs



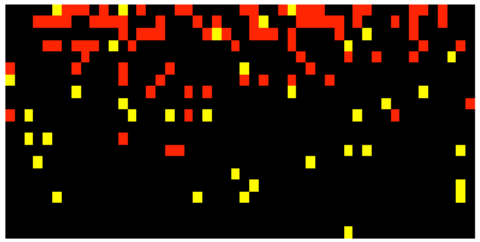
t zero vs 23 hrs



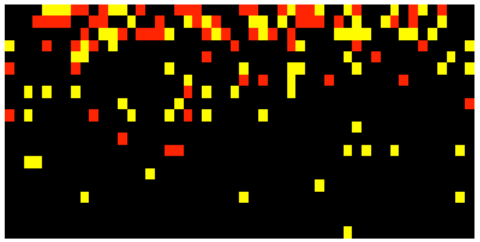
t zero vs 25 hrs



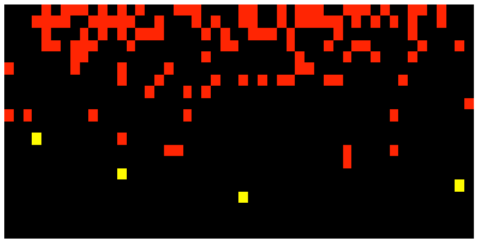
t zero vs 45 hrs



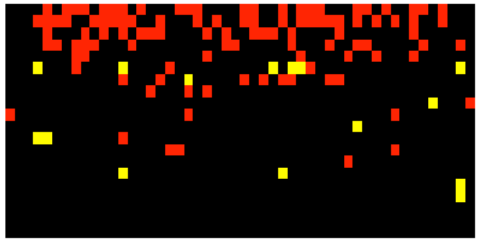
t zero vs 47 hrs



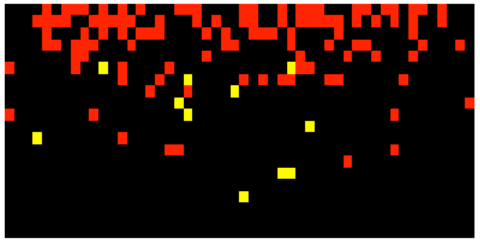
t zero vs 71 hrs



t zero vs 73 hrs



t zero vs 96 hrs



Patterns show changes over five days…

…but can they identify radiation exposure?

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Is it a new biomarker? Or just a face in the clouds?

- *Costs of assays falling*
- *BUT…costs of human and animal studies rising*
- *> 1,000 candidate biomarkers, <100 subjects*

  → high risk of over-fitting data in statistical models

  → false-positive IDs of biomarkers

  - seeing "faces in the clouds"

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A rigorous method for biomarker identification

1. What was the experimental accuracy of a candidate VOC?

Area under curve (AUC) of receiver operating characteristic (ROC curve)

Range: 0.5 (coin-flip) to 1.0 (perfect accuracy)

2. What was its accuracy by chance alone?

Multiple Monte Carlo simulations

Assign random diagnosis (radiation or no radiation)

→ determine AUC of the ROC curve

→ repeat 40 times

→ mean chance accuracy

3. Compare the outcomes

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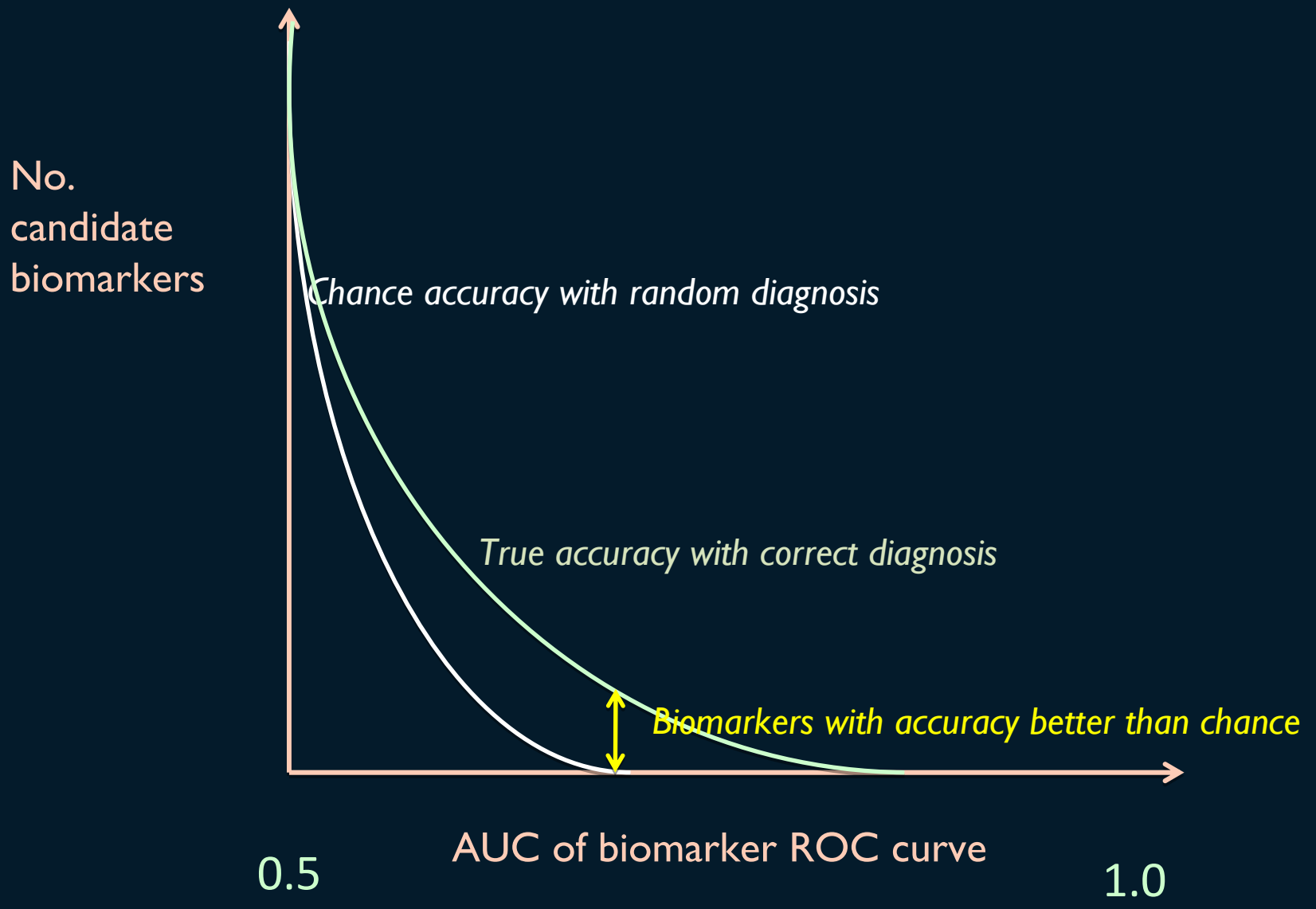

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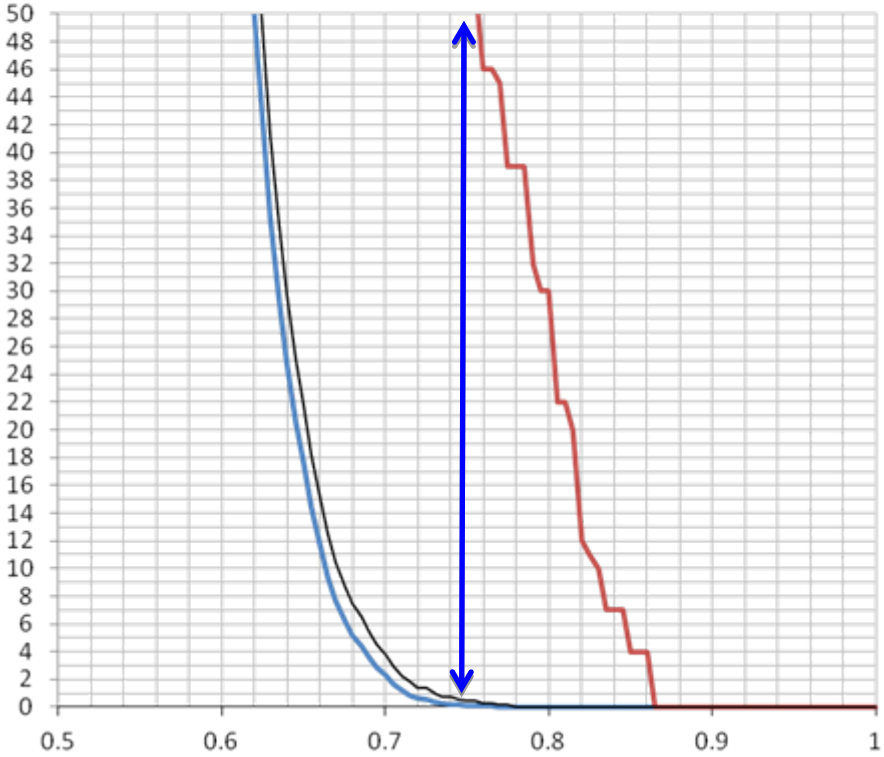
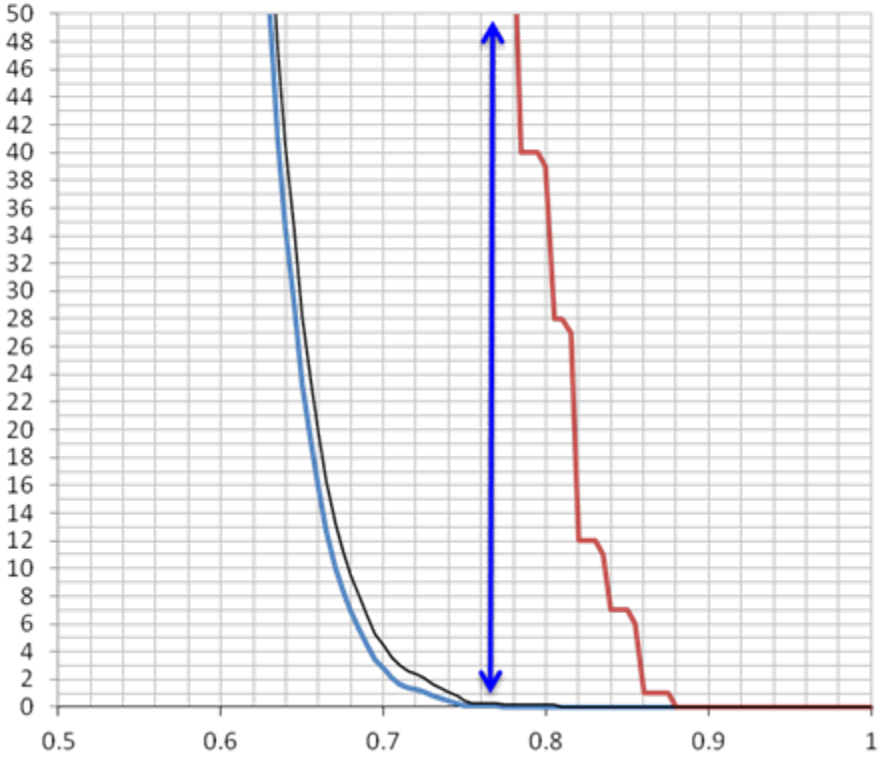
Breath VOCs

Day 1: Baseline pre-radiation vs day 1 post-radiation



Day 5: Baseline pre-radiation vs final breath collection



- *Similar Monte Carlo outcomes at all time points on days 1 through 5*
- *~50 breath VOCs greater than chance at each time point (>5 sigma)*
- *15 recurring breath VOCs seen at all time points*

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15 biomarkers of radiation

Consistently observed at all time points

2,4,4-Trimethyl-3-(3-methylbutyl)cyclohex-2-enone
Cyclononene
1-Methylcyclohexa-2,4-diene
2,4-Octadiene
3-Heptene, 2,2,4,6,6-pentamethyl-
Benzene, (2-methylpropyl)-
Decanoic acid, nonadecafluoro-
Decane, 2,2,3-trimethyl-
Benzene, 1-cyclopenten-1-yl-
Benzene, (2-methyl-1-propenyl)-
4,5,6,7-Tetramethylphthalide
3-Tetradecene, (Z)-
4-Tetradecene, (E)-
Nitrous Oxide
Tetradecanal

Methylated alkane – oxidative stress product

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2,4,4-Trimethyl-3-(3-methylbutyl)cyclohex-2-enone
Cyclononene
1-Methylcyclohexa-2,4-diene
2,4-Octadiene
3-Heptene, 2,2,4,6,6-pentamethyl-
Benzene, (2-methylpropyl)-
Decanoic acid, nonadecafluoro-
Decane, 2,2,3-trimethyl-
Benzene, 1-cyclopenten-1-yl-
Benzene, (2-methyl-1-propenyl)-
4,5,6,7-Tetramethylphthalide
3-Tetradecene, (Z)-
4-Tetradecene, (E)-
Nitrous Oxide
Tetradecanal

Can these biomarkers provide a radiation biodosimeter?

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Dose-response curve

Correlate abundance of 15 biomarker VOCs with total dosage (Gy)

third order polynomial regression

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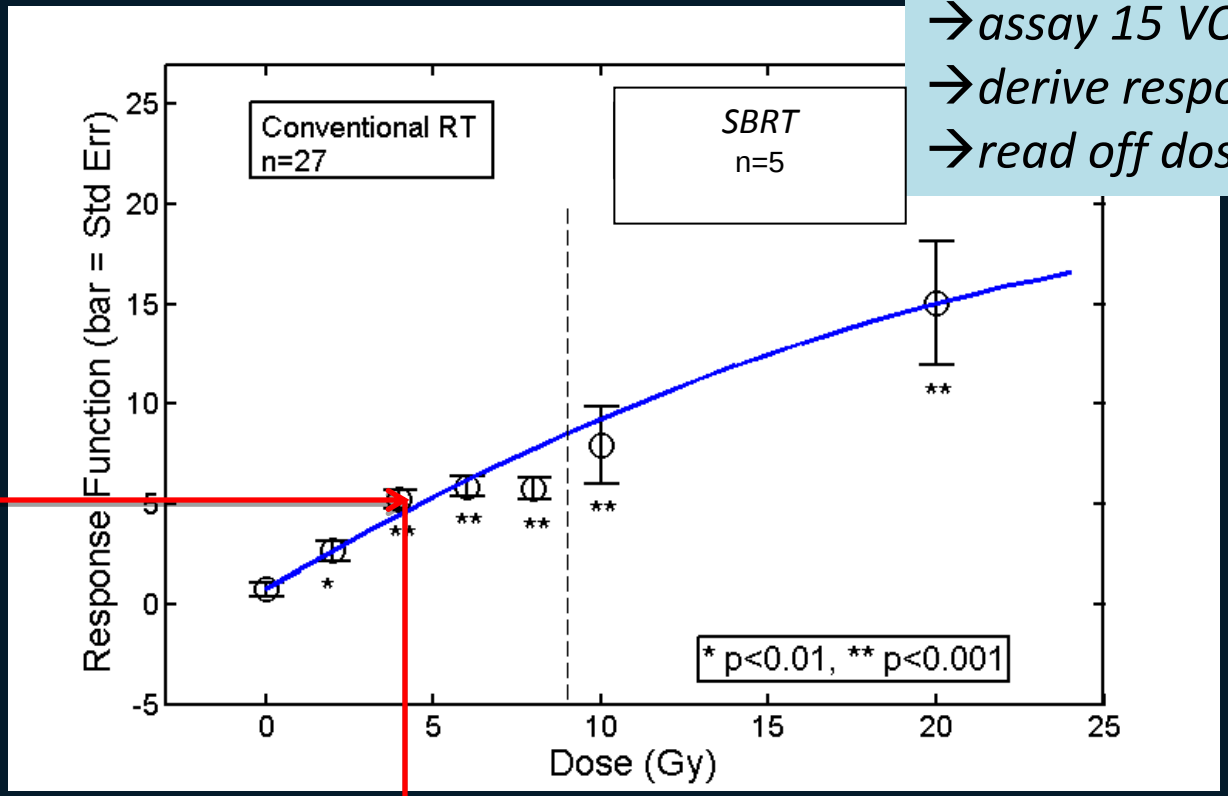
Potential application as a biodosimeter

Collect breath sample
→assay 15 VOC biomarkers
→derive response function
→read off dose from curve



input response function

output dosage

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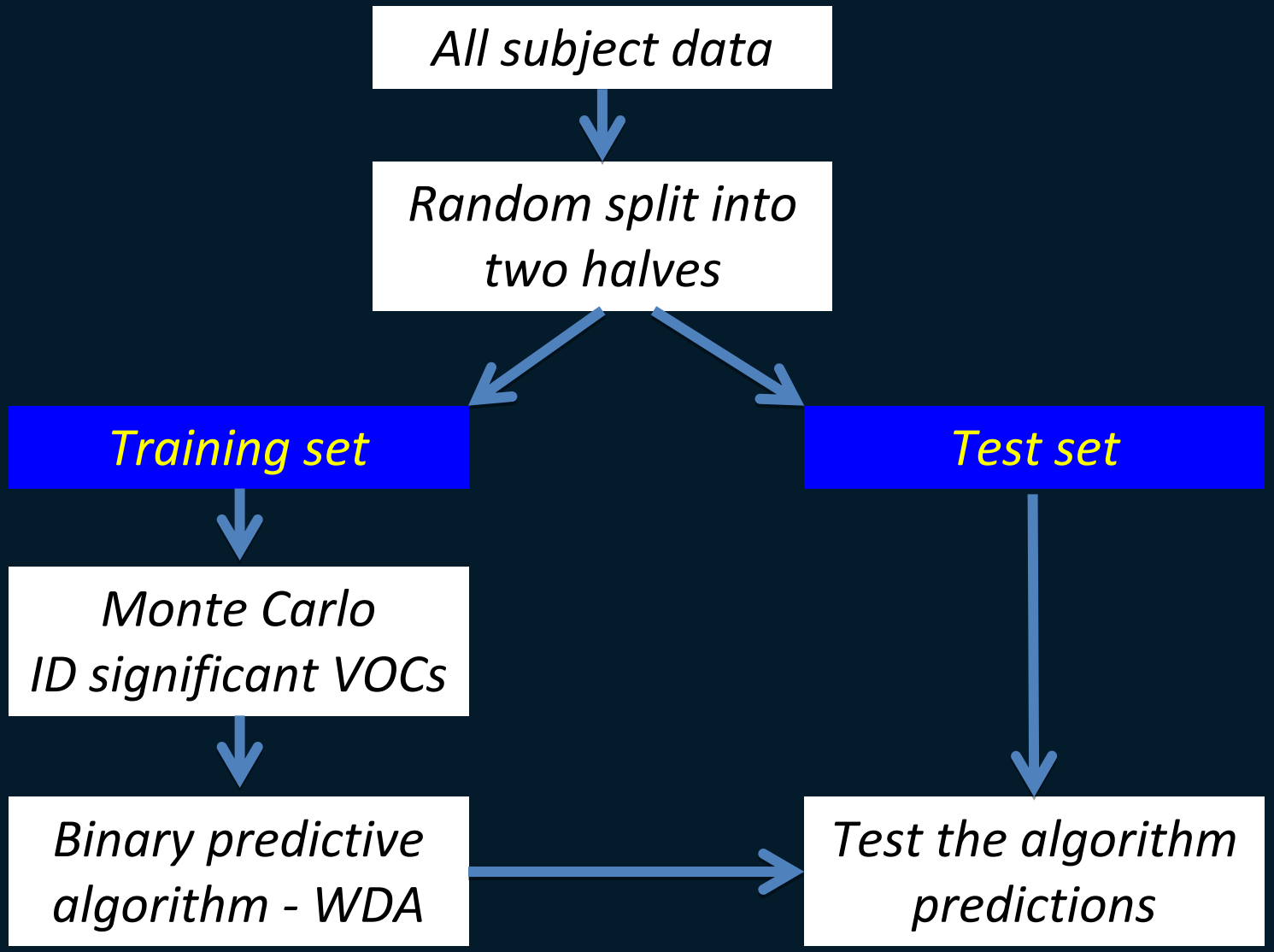
Another approach:
Dose as a binary variable

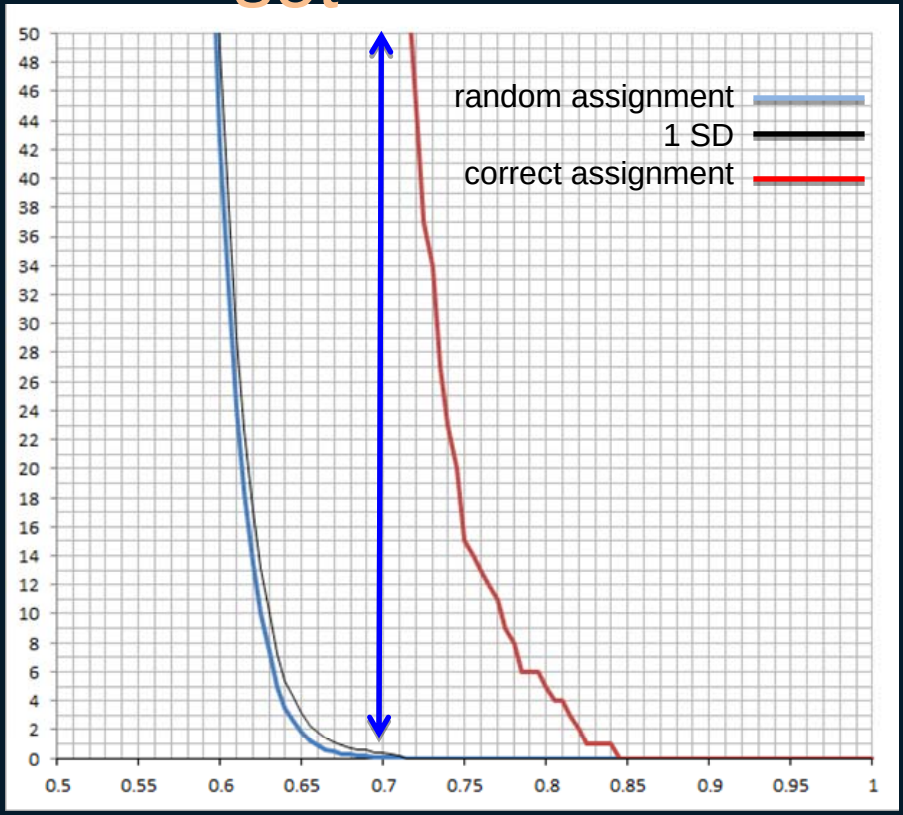
Group 1	Group 2
<1.8 Gy	>= 1.8 Gy

Advantages:

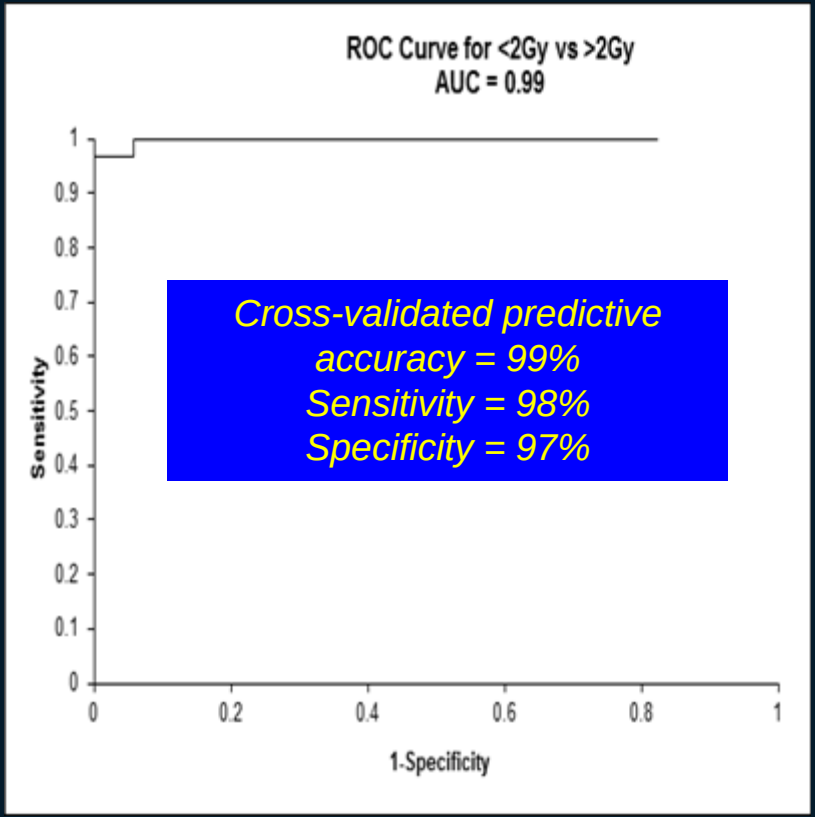
- *Robust binary algorithm*
- *can be cross-validated*


All subject data
Random split into two halves
Training set
Test set
Monte Carlo ID significant VOCs
Binary predictive algorithm - WDA
Test the algorithm predictions

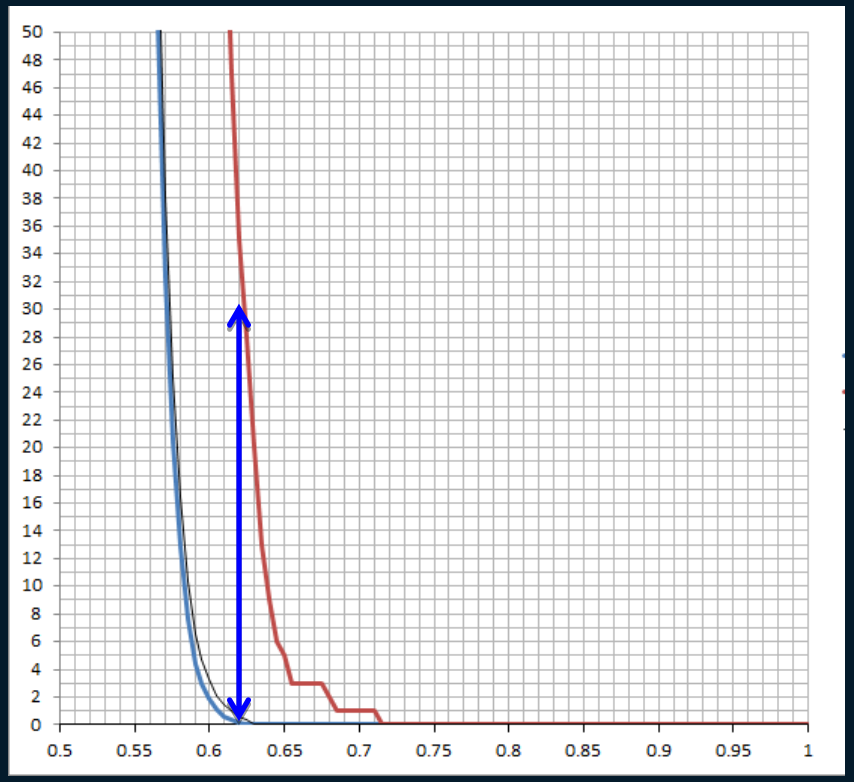
Training set

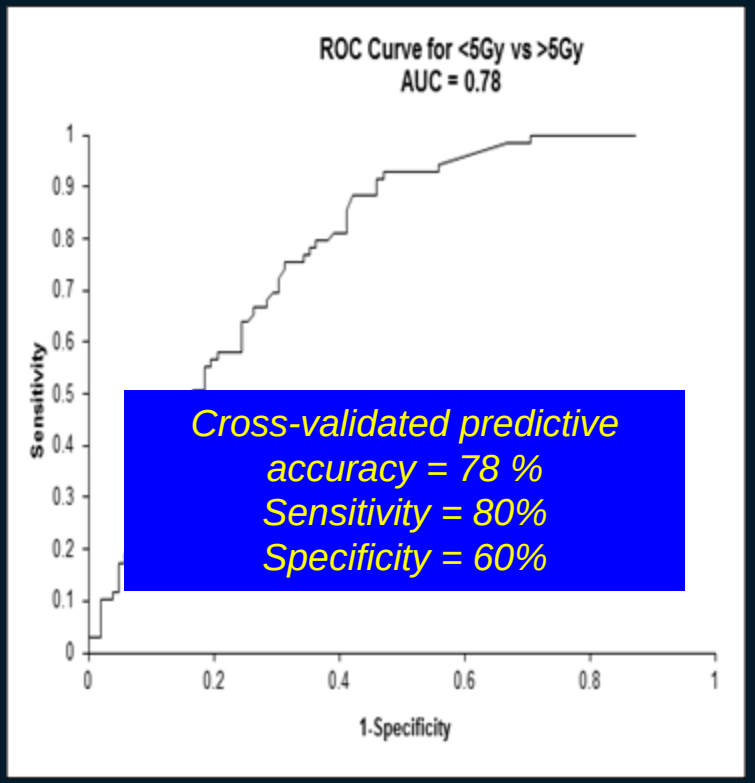


Test set



Group 1	Group 2
<5 Gy	>= 5 Gy

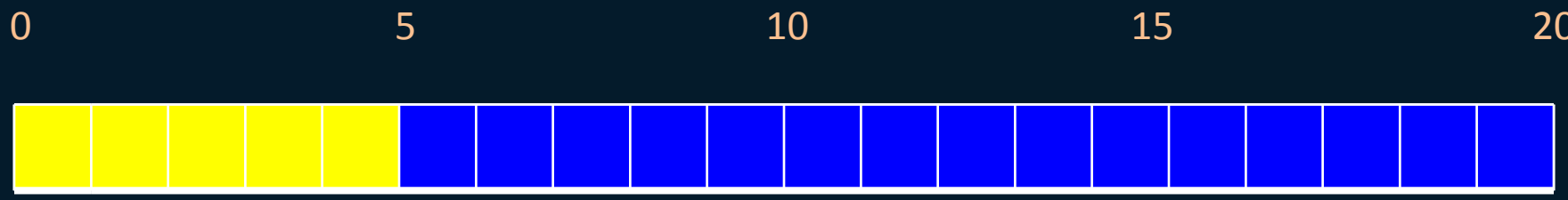




Training set

Test set

Binary radiation biodosimeter



Cutoff at 1.8 Gy
YES/NO accuracy
= 99 %

Cutoff at 5 Gy
YES/NO accuracy
= 78 %

Conclusions

In 32 humans irradiated over 4-5 days:

- *>50 breath VOCs changed significantly (>5 sigma)*
- *15 consistent biomarker VOCs at all time points*
  - *included oxidative stress products*
- *Continuous dose-effect curve*
  - *progressive increments through dose range 1.8 - 20 Gy*
  - *changes significant at all doses >=1.8 Gy*
- *Binary dose-response*
  - *Accurate YES/NO responses at 1.8 Gy and 5 Gy cutoffs*

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Next steps

Currently:

- *Analyzing data - completed animal study*
  - *Irradiated Gottingen minipigs*

Near future:

- *Total body irradiation studies*
  - *Humans*
  - *Non-human primates*

Longer term:

- *Transfer algorithms to point-of-care breath test*
  - *→ rapid cost-effective biodosimeter*

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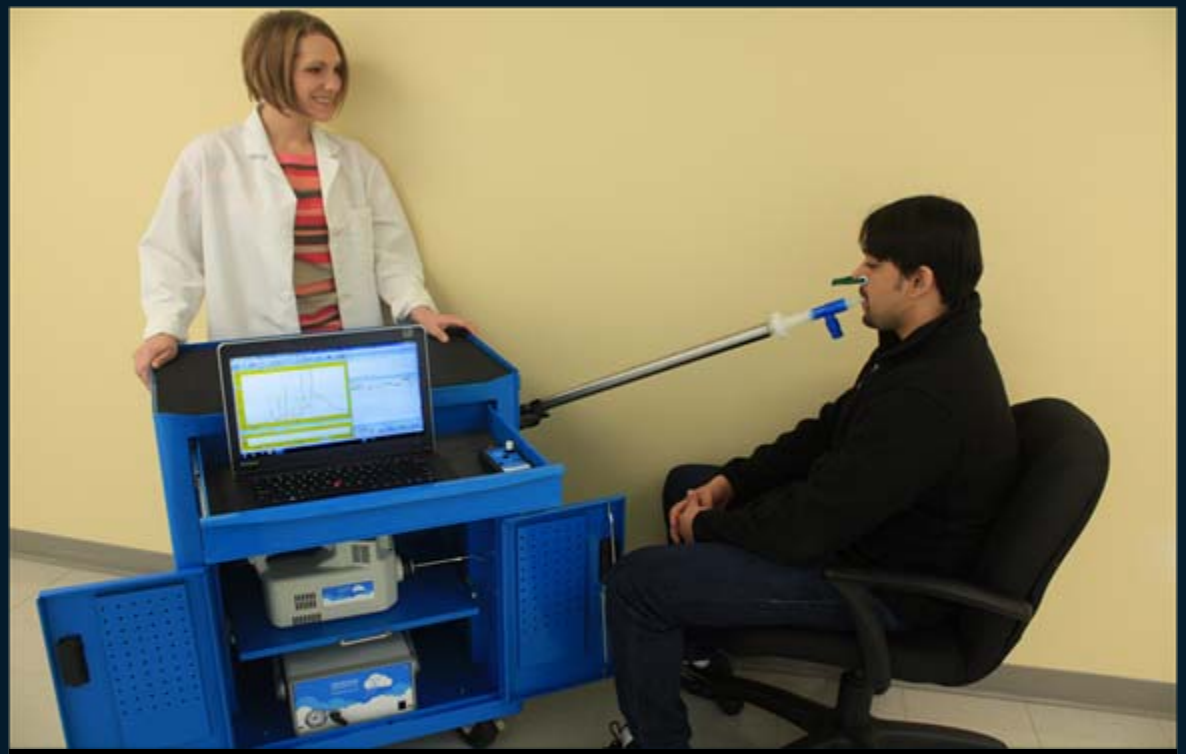
BreathLink
Point-of-care system

Current BreathLink system

Collects and concentrates breath and air VOCs
Separates VOCs (1D GC)
Detects VOCs (SAW)
Assay in <6 min
Detects active pulmonary TB

Next-generation BreathLink

2D POC GC x GC
Smaller, more portable Will detect biomarkers identified by GCxGC TOF MS Optimized for first-responders and triage



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Simple color-coded output for first responders

→ *Rapid triage following a nuclear event*

Thanks for listening!